Epilepsy & Behavior 78 (2018) 142–148

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Modulation of the anticonvulsant effect of swim stress by agmatine

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ARTICLE INFO

Article history:
Received 16 July 2017
Revised 29 October 2017
Accepted 5 November 2017
Available online xxxx

Keywords:
Agmatine
Pentylenetetrazole-induced clonic seizures
Naltrexone
Nitric oxide
Mice

ABSTRACT

Agmatine is an endogenous L-arginine metabolite with neuroprotective effects in the stress-response system. It exerts anticonvulsant effects against several seizure paradigms. Swim stress induces an anticonvulsant effect by activation of endogenous antiseizure mechanisms. In this study, we investigated the interaction of agmatine with the anticonvulsant effect of swim stress in mice on pentylenetetrazole (PTZ)-induced seizure threshold. Then we studied the involvement of nitric oxide (NO) pathway and endogenous opioid system in that interaction. Swim stress induced an anticonvulsant effect on PTZ seizures which was opioid-independent in shorter than 1-min swim durations and opioid-dependent with longer swims, as it was completely reversed by pretreatment with naltrexone (NTX) (10 mg/kg), an opioid receptor antagonist. Agmatine significantly enhanced the anticonvulsant effect of opioid-independent shorter swim stress, in which a combination of subthreshold swim stress duration (45 s) and subeffective dose of agmatine (1 mg/kg) revealed a significantly higher seizure threshold compared with either one. This effect was significantly reversed by NO synthase inhibitor N^G-nitro-L-arginine (L-NAME (Nω-Nitro-L-arginine methyl ester), 5 mg/kg), suggesting an NO-dependent mechanism, and was unaffected by NTX (10 mg/kg), proving little role for endogenous opioids in the interaction. Our data suggest that pretreatment of animals with agmatine acts additively with short swim stress to exert anticonvulsant responses, possibly by mediating NO pathway.

1. Introduction

Agmatine is an endogenous guanidoamine compound produced from L-arginine by arginine decarboxylase. It has been shown that many organs, including brain, contain agmatine. Studies suggest that agmatine could be regarded as a neurotransmitter or a cotransmitter since it is stored in vesicles of neurons, and reuptake inactivates it. Moreover, agmatine is degraded by its specific enzyme agmatinase and released by depolarization as a result [1]. It binds to α2-adrenoceptors, imidazoline-binding sites, as well as N-methyl-D-aspartate (NMDA) receptors [2,3]. Agmatine is believed to be involved in stress-response system as a protective factor and is known to exert neuromodulatory and neuroprotective actions in ischemia, stress, and different pain models [4–7]. Its level rises in plasma and some brain areas of rats in response to stress [8]. Agmatine effectively inhibits the lipopolysaccharide-induced fever and restraint stress-induced hyperthermia in rats [9]. It exerts anxiolytic effects in mice and rats [10] and also decreases the depressive-like behavior induced by acute restraint stress and force swim stress tests in mice [11].

The antiseizure activity of agmatine has received a lot of recent attention because of its therapeutic potentials. Our previous work showed that agmatine exerted a dose-dependent anticonvulsant effect against the seizures induced by pentylenetetrazole (PTZ) in mice, a model for myoclonic seizures [12]. Similarly, other labs have reported an anticonvulsant effect for agmatine against maximal electroshock-induced seizures, a model for generalized tonic–clonic seizures, in rats [13] and in mice [14] as well as in other seizure models [14,15]. Reports show that agmatine is capable of potentiating the antiseizure effects of several other chemicals including morphine [16], lithium chloride [17], melatonin [18], and antiseizure medications, phenobarbital and valproate [19]. The mechanism for the antiseizure activity of agmatine has been attributed to several of its actions including inhibition of NO production [20], antagonism of NMDA receptors [3,21], activation of α2-adrenoceptors [12,16], and lowering the extracellular glutamate content [22]. Agmatine selectively inhibits different subtypes of nitric oxide synthase (NOS) in the central nervous system (CNS) [1]. However, the complete mechanism of its effect is yet to be known.

In this work, we studied the interaction of agmatine with the anticonvulsant effect of swim stress in mice. Swim stress induces an anticonvulsant effect by activation of endogenous antiseizure

Abbreviations: PTZ, pentylenetetrazole; L-NAME, L-N^G-nitroarginine methyl ester; CST, clonic seizure threshold.

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https://doi.org/10.1016/j.yebeh.2017.11.005
1525-5050/

mechanisms, for instance, through elevation in brain concentrations of γ-aminobutyric acid class A (GABA-A) receptor active neurosteroids [23,24]. Agmatine has been previously shown to enhance the antinociceptive effect of swim stress [25] possibly implying that agmatine might also interact with its anticonvulsant effect. Then, we examined the possible involvement of endogenous opioid system and nitric oxide pathway in that interaction. Opioid receptors have been shown to participate in the antinociceptive effects of swim stress [26]. Thus, taking into account the considerable amount of existing data regarding the interaction of agmatine with several effects of opioids, including enhancing their analgesic [27,28], anticonvulsant [16], and rewarding properties [29], as well as reducing the development of tolerance and dependence to their effects [30,31], we asked if the involvement of the endogenous opioid system might be a potential underlying mechanism for the effect of agmatine on the anticonvulsant effect of swim stress. Finally, previous research has proven that NOS inhibition potentiates the swim stress-induced antinociception in rats [32]. Therefore, a role for NO pathway in mediating the interaction of agmatine with swim stress in our seizure paradigm seemed plausible. The method that we used to measure convulsive tendency was the threshold of seizures induced by timed intravenous infusion of PTZ, a γ-aminobutyric acid (GABA) transmission blocker, which is a sensitive and established model for clonic seizures in mice [33].

2. Methods and materials

2.1. Animals

Male Swiss mice (Razi Institute, Iran) weighing 25–35 g were used in the study. The animals were housed at constant room temperature (25 °C) and submitted to 12-h light, 12-h dark cycle with free access to food and water. They were housed in standard polycarbonate cages and acclimated at least 2 days before experiments. Each treatment group consisted of 5–9 animals. Experiments were performed in accordance with the Animal Experiments recommendations of the Ethics Committee of the School of Medicine, Tehran University of Medical Sciences.

The study procedures were approved by the ethics committees of the Department of Pharmacology, Deputy Office for Research and Technology of Tehran University of Medical Sciences and the ethics committee of the School of Pharmacy.

2.2. Drugs

Naltrexone (NTX), an opioid antagonist, was a generous gift from Iran Daru (Tehran, Iran). Pentylenetetrazole (PTZ), L-NAME (N_{ω}-Nitro-L-arginine methyl ester), and agmatine were purchased from Sigma (UK). L-Arginine was purchased from Fluka (Switzerland). All drugs were freshly prepared in saline solution to such concentration that the required doses were administered in a volume of 10 ml/kg. Pentylenetetrazole solution was prepared in saline as 1% solution and was given intravenously through a tail vein, while other drugs were injected intraperitoneally (i.p.). All experimental testing sessions were conducted between 9.00 A.M. and 6:00 P.M.

2.3. Swim stress procedure

The procedure was similar to that described by Porsolt et al. with some modifications [34]. A cylinder of larger diameter was used in order to increase predictive validity in the mouse swim test [35]. To facilitate adaptation to novel surroundings, mice were transported to the testing room at least 1 h prior to test. Mice were placed in plexiglas cylinder (45 cm tall and 20 cm diameter) containing 18–19 °C water, 15 cm deep. The temperature was chosen based on existing literature [36–38] measured and adjusted before each animal. After specific swimming duration, the animals were gently dried with a towel and placed near a heater. The seizure thresholds were measured 15 min after the termination of stress. Nonstressed control animals were taken for comparison. The control mice were similarly transported to the test room 1 h prior, gently massaged with a towel, and were placed near the heater for 15 min before clonic seizure threshold (CST) testing. In order to minimize the possibility of differences in the body temperatures between swim stressed animals and nonstressed controls when CST was being tested, we used large cages for both groups and put the heater on one side of the cage to allow the animals to move away from the heater to avoid hyperthermia.

2.4. PTZ-induced seizure threshold measurement

To determine the threshold for PTZ-induced clonic seizures, mouse was placed in a restrainer, and its lateral tail vein was cannulated by a 30-gauge dental needle connected by polyethylene tubing to a Hamilton micro syringe containing 1% PTZ solution. The needle was then fixed with a piece of adhesive tape. Then, the animal was released from the restrainer and while moving freely, PTZ was infused at a constant rate of 0.6 ml/min using an infusion pump. Minimal dose of PTZ (calculated as milligrams per kilogram of mice body weight) needed to induce general clonus was used as the indicator for the CST. The general clonus was characterized by forelimb clonus followed by transient loss of righting reflex to full clonus of the body [16].

2.5. Experiments

In experiment 1, we studied the effect of agmatine on CST. Animals had been given either saline as control or different doses of agmatine (1, 2, 5, 10, 20 mg/kg) 45 min before they underwent the measurement of CST. The 45-min time-point was chosen based on a time-course experiment from our previous study in which agmatine had a maximum effect at 30–45 min after injection [12,17]. In experiment 2, we assessed the effect of agmatine on the anticonvulsant effect of swim stress. Two groups of mice received i.p. injections of either saline as controls or a subeffective dose of agmatine (1 mg/kg, from experiment 1), 15 min before being subjected to swim stress. Clonic seizure threshold measurements were performed 15 min after the termination of stress.

Experiments 3 and 4 were performed to test the possible role of endogenous opioids in the interaction of agmatine with the anticonvulsant effect of swim stress. First, we tested the involvement of endogenous opioids in the increase of CST caused by swim stress. Animals received i.p. injections of either NTX (10 mg/kg) or equal volume of saline as control, 30 min prior to swim stress, and then CSTs were measured 15 min after the termination of stress. Clonic seizure thresholds were measured in separate groups of animals from either treatment after different durations of swim stress ranging from 30 s to 10 min. Then we tested if the enhancing effect of agmatine on the anticonvulsant effects observed with short-duration swim stress was related to its enhancement of opioid effects. We compared four groups of animals. They received either saline or NTX (10 mg/kg), followed by either saline or agmatine (1 mg/kg) 15 min later. Then all 4 groups were subjected to 45 s of swim stress 15 min after the second injections and then underwent CST measurement 15 min after the swim stopped. The outcome was to see if the effect of agmatine would be reversed by NTX pretreatment.

In experiment 5, we tested the role of NO system in CST modulation by swim stress. L-NAME, a nonspecific NOS inhibitor (5 mg/kg, i.p.), L-arginine, an NO precursor (100 mg/kg, i.p.), or saline was administered to nonstressed animals or 30 min before 1 min of swim stress followed by CST testing 15 min later. The doses of L-NAME and L-arginine were selected from our previous studies and our pilot experiments as the highest dose incapable of exerting effect on CST [20,39]. The thresholds in nonstressed animals were tested 46 min after the injections to match with the stressed groups. In experiment 6, we checked if the interaction of agmatine with swim stress on CST was resulted from NO pathway modulation. Four groups of animals were tested. The first